

# Models of 2,4,6-trinitrotoluene (TNT) initial conversion by yeasts

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Received 31 July 2002; received in revised form 4 October 2002; accepted 16 October 2002

First published online 6 November 2002

## Abstract

The original models of the initial steps of 2,4,6-trinitrotoluene (TNT) conversion by yeasts are presented. *Saccharomyces* sp. ZS-A1 reduced nitro groups of TNT producing isomeric monohydroxylaminodinitrotoluenes (HADNT) as the key initial metabolites (molar ratio HADNT/TNT was up to 0.81), whereas aminodinitrotoluenes (ADNT) and the hydride–Meisenheimer complex of TNT (H-TNT) were the minor products. Conversely, *Candida* sp. AN-L13 transformed TNT almost quantitatively into H-TNT, thus realizing the alternative attack, consisting of the TNT aromatic ring reduction. The third type of conversion, revealed in *Candida* sp. AN-L14, is the combination of both above mechanisms and produces an equimolar mix of HADNT and H-TNT. In the toxicity tests with *Paramecium caudatum*, the supernatant of *Saccharomyces* sp. ZS-A1, which converts TNT into HADNT, was most toxic while the supernatant of *Candida* sp. AN-L13 (TNT→H-TNT) was least toxic. The microorganisms converting TNT quantitatively to the reactive metabolites can be useful for their immobilization through the detoxifying interaction with the soil components such as humic compounds.

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**Keywords:** 2,4,6-Trinitrotoluene; Initial transformation; Reactive metabolite; Yeast; Toxicity; Explosives and oil contamination

## 1. Introduction

The widespread dissemination of the nitroaromatic compounds in the environment is due to their use as pesticides, in the production of chemical precursors, explosives, and a number of other applications. 2,4,6-Trinitrotoluene (TNT) is a major component of military and industrial explosives. It is well known that technological operations related to its production, storage, and use result in a widespread dispersion of TNT in the environment as a characteristic pollutant. For example, in Germany, where TNT was the main explosive produced during World War II, in about 60 former production sites TNT may persist in the solid state and act as a long-term source of soil and groundwater contamination [1]. The presence of TNT in the polluted areas, decades after the halting of its production, suggests that there are metabolic barriers

preventing the biodegradation and entrance of this pollutant into the global biogeochemical carbon and nitrogen cycles. The existence of such barriers is also substantiated by the poor mineralization rate of TNT and its metabolites in different composting systems [2].

In TNT biotransformation, the most commonly detected metabolites are aminodinitrotoluenes (ADNT) and, in some cases, diaminodinitrotoluenes (DANT) [3–7]. On the other hand, more recent investigations have discovered the early reduced metabolites of TNT, namely hydroxylaminodinitrotoluenes (HADNT), which are the products of four-electronic reduction of the 2- or 4-nitro groups by fungi [8] and bacteria [9–11]. Some lactobacilli are able to transform up to 600  $\mu\text{mol}$  of TNT stoichiometrically to HADNT [9,12]. It has been shown that a partially reduced alternative intermediate, the hydride–Meisenheimer complex of TNT (H-TNT), can be produced by some bacteria through the transfer of hydride ion to the aromatic ring of TNT [14].

While this restricted reductive conversion of TNT has been demonstrated for some bacteria, actinomyces, and fungi [14,15], the possibility of analogous conversion by yeasts has not been elucidated. Thus the aim of this

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